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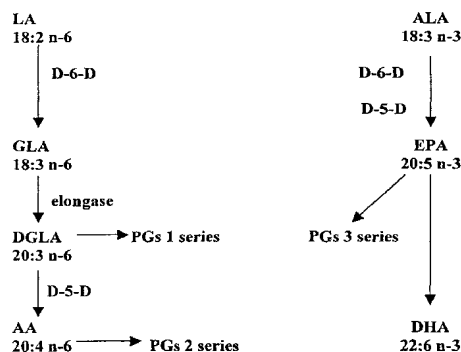
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(54) Title: COMPOSITION FOR STABILIZING AND POTENTIATING THE ACTION OF ANTI-ANGIOGENIC SUBSTANCES BY POLYUNSATURATED FATTY ACIDS



SHOWING THE METABOLISM OF ESSENTIAL FATTY ACIDS

(57) Abstract: A method of stabilizing and potentiating action of molecules of known anti-angiogenic substances such as Angiostatin[®] or Endostatin[®] by using in coupling conjugation with cis-unsaturated fatty acids (c-UFAs) in the treatment of cell proliferative disorders uses c-UFAs chosen from linoleic acid, gamma-linolenic acid, dihomo-gamma-linolenic acid, arachidonic acid, alpha-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid and cisparinaric acid in predetermined quantities. Preferably, the c-UFAs are in the form of polyunsaturated fatty acids (PUFAs). Uncontrolled or undesirable angiogenic activity promotes cell proliferative disorders and tumor growth, which can be inhibited by the selective use of PUFAs with anti-angiogenic substances used selectively in conjunction with predetermined anti-cancer drugs. For treatment of glioma, a sodium salt of a PUFA is preferred to form an admixture with an anti-angiogenic substance and a selected anti-cancer drug. For a non-glioma type of cell proliferation disorder, a sodium, potassium or lithium salt of a PUFA is preferred to form an admixture with an anti-angiogenic substance. Anti-angiogenic substances envisaged in this invention include Angiostatin[®], Endostatin[®], platelet factor-4, TNP-470, thalidomide, interleukin-12 and metalloproteinase inhibitors (MMP). A preferred method of administration of the mixture to treat a tumor is intra-arterial administration into an artery which provides the main blood supply for the tumor.



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COMPOSITION FOR STABILIZING AND POTENTIATING THE ACTION OF ANTI-ANGIOGENIC
SUBSTANCES BY POLYUNSATURATED FATTY ACIDS

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Field of the Invention :

The present invention generally relates to the use of anti-angiogenic agents
15 in the cure of cell proliferative disorders including cancer and other
disorders caused by uncontrolled angiogenic activity in the body. More
particularly, the invention is directed to the efficacious use of anti-
20 angiogenic agents.

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Related Applications:

This invention relates to copending U.S. application Serial No. 09/392,953
Filed on September 9, 1999 and entitled “ Method of Treatment for Cell
30 Proliferative Disorders including Cancer”, which is incorporated herein by
reference.

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5 **Background of the Invention :**

10 The term angiogenesis refers to the generation or formation of new blood
vessels into a tissue or organ. Angiogenesis can occur both during some
physiological processes and/or in some pathological conditions. For
15 example, angiogenesis can be seen to occur during wound healing, fetal
growth, corpus luteum, and endometrium, etc., (1). Endothelial cells, which
cause to form the inner lining of the blood vessels, are constituted by a thin
20 layer of epithelial cells and these cells are necessary for the process of
angiogenesis. During the process of angiogenesis, irrespective of whether it
is physiological or pathological, the endothelial cells release enzymes which
25 can produce erosions of the basement membrane through which the
endothelial cells cause protrusions. In response to the stimuli given by
30 various agents, endothelial cells proliferate and migrate through the protru-
sions and form a sprout of the parent blood vessel. These endothelial cell
sprouts can merge to form capillary loops leading to the formation of new
35 blood vessel(s). If the blood vessels are in a tumor area, these new

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blood vessels in turn will provide enough nutrients and energy sources
so that tumor cells can divide, proliferate and grow both in number and size.
10 Thus, the process of angiogenesis is both essential and critical to the growth
of cancer. The other pathological states in which angiogenesis plays a
15 critical role include: rheumatoid arthritis, psoriasis, scleroderma, myocardial
angiogenesis, corneal diseases, diabetic retinopathy associated with
neovascularization, macular degeneration, ovulation, menstruation etc. The
20 process of angiogenesis also appears to be critical for tumor metastasis.

Since angiogenesis is such a critical process in the promotion of cancer
25 and tumor metastasis, several researches have been trying to devise methods
or develop drugs which can selectively suppress angiogenesis with the hope
30 that this would eventually lead to the inhibition of tumor growth. There are
other situations where uncontrolled angiogenesis is undesirable. For
instance, formation of new blood vessels in an area like cornea during the
35 process of healing of the corneal ulcer, if it is in excess, can lead to corneal
scar formation.

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In the case of rheumatoid arthritis, angiogenesis can lead to continued inflammation in the joints and also to osteoporosis. In such an instance, prevention of formation of new blood vessels will lead to reduction in inflammation and also prevention of fibrous ankylosis and bony ankylosis. Thus, selective prevention and control of angiogenesis may be of benefit in the aforementioned conditions, as well as in several other conditions such as: uterine fibroids, psoriasis, scleroderma, diabetic retinopathy, keloids, ovulation etc. Another area where prevention of angiogenesis will be of benefit is in the inhibition of ovulation and menstruation and growth of placenta and this will lead to prevention of fertilization and growth of the fetal tissue. This may, thus, form a new approach in the development of fertility control measures.

Two naturally occurring molecules which have been identified to adversely influence or inhibit angiogenesis are angiostatin[®] and endostatin[®] (2). Both these molecules are proteins. Angiostatin[®] is a protein of molecular weight approximately 38 kD and has an amino acid sequence substantially similar to that of a fragment of murine plasminogen beginning

at amino acid number 98 of an intact murine plasminogen molecule. The amino acid sequence of angiostatin[®] varies only slightly between species.

5 The amino acid sequence of the human angiostatin is substantially similar to the murine plasminogen fragment. But, it may be mentioned here that the active human angiostatin sequence starts either at the amino acid number 97
10 or 99 of an intact human plasminogen amino acid sequence. In addition, human plasminogen has potent anti-angiogenic activity even in a mouse tumor model. This explains why both murine and human plasminogens and
15 angiostatin[®]/endostatin[®] molecules show fairly similar anti-angiogenic activities in a variety of animal tumor models (3).

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U. S. patent 5, 792,845 issued on August 11, 1998 to O'Reilly et al teaches that therapies directed at control of the angiogenic process could
25 lead to the abrogation or mitigation of certain diseases. O'Reilly et al suggests that modulation of the formation of capillaries in angiogenic processes (such as wound healing and reproduction) is useful since
30 undesired and uncontrolled angiogenesis can cause certain diseases to progress. O'Reilly et al teaches that angiostatin[®] protein has the capability of
35 inhibiting angiogenesis, eg., to inhibit the growth of bovine capillary endothelial cells in culture in the presence of fibroblast growth factor.

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U.S. patent 5,932,545 issued on August 3, 1999 to Henkin et al teaches an anti-angiogenic drug in the form of a peptide or a salt thereof, to treat
5 cancer, arthritis and retinopathy. The Henkin et al patent states however that angiogenesis inhibitors could cause systemic toxicity in humans.

10 Angiostatin[®] in the O'Reilly patent '845 is described and claimed as an Isolated nucleotide molecule with a specific sequence. It has been stated however that the angiostatin[®] molecule as known at present is not suitable
15 for clinical trials.

Endostatin[®], which is also similar to angiostatin[®], has been shown to
20 cause a dramatic reduction of primary and metastatic tumors in experimental animals. Endostatin[®] is a 20 kDa C-terminal fragment of collagen XVIII. Endostatin[®] could specifically inhibit endothelial cell proliferation and
25 angiogenesis and thus, block tumor growth (2, 4).

30 It is important to note that angiostatin[®] is derived from plasminogen or plasmin. It has been shown that human prostate carcinoma cell lines express enzymatic activity that can generate bioactive angiostatin[®] from purified
35 human plasminogen or plasmin. This bioactive angiostatin[®] has been shown to inhibit human endothelial cell proliferation, basic fibroblast growth
40 factor-induced migration, endothelial cell tube formation, and basic

fibroblast growth factor-induced corneal angiogenesis. In an extension of
this study, it was noted that a serine proteinase is necessary for angiostatin®
5 generation (5).

Angiostatin®, derived from plasminogen, selectively inhibits endothelial
10 cell proliferation. When angiostatin® is given systemically it shows potent
inhibitory action on the growth of tumor and renders metastatic and primary
15 tumors to go into a dormant state by striking a balance between the rate of
proliferation and apoptosis of the tumor cells (6). The very identification of
angiostatin® has come from the observation that when a primary tumor is
20 present, the growth of metastases is suppressed. On the other hand, after
tumor removal, the previously dormant metastases develop new blood
25 vessels (neovascularization) and grow. Both serum and urine from the
tumor-bearing animals, but not from controls, showed very specific
inhibitory action on the growth of endothelial cells. Subsequent studies led
30 to the purification of this inhibitor of endothelial cells which was later
identified as a 38 kD plasminogen fragment namely angiostatin®. It is now
35 known that angiostatin®, which can also be obtained by a limited proteolytic
digestion of human plasminogen, but not intact plasminogen can be
administered systemically to block neovascularization and growth of

metastases and primary tumors. A recombinant human angiostatin[®] which
5 comprises of kringles 1-4 of human plasminogen (amino acids 93-470)
expressed in *Pichia pastoris* has been prepared and is now available for use.
This recombinant angiostatin[®] showed the same physical properties as that
10 of the natural angiostatin[®] in terms of molecular size, binding to lysine,
reactivity with antibody to kringles 1-3 (3, 7). This recombinant
15 angiostatin[®], when given to experimental animals, showed anti-angiogenic
and anti-tumor activity (3). In addition, recombinant mouse angiostatin[®] was
produced using the baculo-virus infected insect cells (8), which also (the
20 secreted protein) showed potent inhibitory action on the proliferation of
bovine capillary endothelial cells in vitro. The conversion of plasminogen to
25 angiostatin[®] by PC-3 cells is now identified to be due to two components
released, urokinase (uPA) and free sulfhydryl donors (FSDs). This is
supported by the fact that even in a cell-free system, angiostatin[®] can be
30 generated from plasminogen by plasminogen activators (u-PA, tissue-type
plasminogen activator, tPA or streptokinase) in combination with any one of
35 free sulfhydryl donors such as N-acetyl-L-cysteine, D-penicillamine,
captopril, L-cysteine, or reduced glutathione. This cell-free derived

angiostatin[®] also showed anti-angiogenic activity both in vitro and in vivo
5 and suppressed the growth of Lewis lung carcinoma metastases (9).

Angiostatin[®] administration to mice with subcutaneous hemangioendo-
10 thelioma and associated disseminated intravascular coagulopathy revealed
that in addition to a significant reduction in the size of the tumor, increased
survival, decrease in thrombocytopenia and anemia was noted (10). This
15 indicates that angiostatin[®] may also be useful to treat disseminated
intravascular coagulopathy.

20 One of the mechanisms by which angiostatin[®] inhibits endothelial cell
proliferation includes its ability to affect by 4 to 5 fold the expression of
25 E-selectin in proliferating endothelial cells (11). On the other hand,
angiostatin[®] did not alter cell cycle progression significantly. Further,
angiostatin[®] also enhanced the adhesion activity in proliferating endothelial
30 cells.

Rivas et al (12) studied the possible relationship between human
35 macropahge metalloelastase (HME) expression, a member of the human
matrix metalloproteinase family, which is believed to play an important role

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in angiostatin[®] generation, and angiostatin[®] production. Their study showed
5 that patients whose tumors did not express HME mRNA and so did not
produce angiostatin[®], had poorer survival than those whose tumors showed
10 high expression of HME mRNA and angiostatin[®] generation. This study
suggests that HME gene expression is closely associated with angiostatin[®]
generation and prognosis in patients with hepatocellular carcinoma (HCC).
15 This relationship between HME and angiostatin[®] is understandable since,
metalloproteinase(s) can block angiogenesis by converting plasminogen to
20 angiostatin[®] (12,13,14).

Another mechanism by which recombinant human and murine
25 angiostatins can block angiogenesis is by inducing apoptosis (programmed
cell death) of endothelial cells (15), similar to that seen with tumor necrosis
factor (TNF) and transforming factor-beta 1 (TGF-beta1), which are also
30 known to induce apoptosis in endothelial cells.

Yet another mechanism by which angiostatin can produce apoptosis and
35 inhibit angiogenesis is probably by binding to ATP synthase. Using human
umbilical endothelial vein endothelial cells, Moser et al (16) observed that
40 angiostatin[®] bound in a concentration –dependent, saturable manner to the

alpha/beta sub-units of ATP synthase. This binding of angiostatin[®] to the
5 alpha/beta sub-unit of ATP synthase was inhibited by as much as 90% in the
presence of anti-alpha-sub-unit ATP synthase antibody. This indicates that
angiostatin[®] by binding to ATP synthase may actually shut-off ATP
10 synthesis in the endothelial cells and this would eventually lead to death of
the cells due to the non-availability of ATP, the main energy source for the
survival of the cells. In addition, it was also reported that angiostatin can
inhibit extra-cellular-matrix-enhanced, t-PA catalysed plasminogen
activation. This results in reduced invasive activity of endothelial cells (17).
20 All these results indicate that angiostatin[®] has multiple actions by which it
is able to block endothelial cell proliferation and angiogenesis.

25 Some of the factors which are known to inhibit the generation of
angiostatin[®] include TGF-beta1 and plasminogen activator inhibitor type-1
30 (PAI-1), at least, by human pancreatic cancer cells in vitro (18).

Twining et al (19) showed that plasmin, the active form of plasminogen,
35 is necessary for the maintenance of normal cornea and for corneal wound
healing. It was also noted that plasmin is a major serine proteinase in the
human cornea and that cornea can synthesize plasminogen. Both interleukin-
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1alpha and 1 beta stimulated corneal plasminogen synthesis by almost 2 to 3
5 fold where as interleukin-6 decreased corneal plasminogen synthesis by
40%. Thus, cornea seems to have the ability to synthesize plasminogen, the
precursor of plasmin and angiostatin[®], and also regulate its synthesis in
10 response to injury and inflammation and IL-1 and IL-6 (19).

Though both angiostatin[®] and endostatin[®] and other similar anti-
15 angiogenic molecules provided an important therapeutic advance for cancer
treatment, it should be emphasized here that the needed dosages of these
proteins, especially angiostatin[®] used in the animal studies seem to be too
20 high for clinical trials (20). Further, repeated injections and long-term
treatment with angiostatin[®] are required to obtain its maximal anti-tumor
25 effect. In view of this, methods to supplement the anti-angiogenic action of
angiostatin and endostatin and other similar compounds are considered
desirable. These methods include: use of angiostatin along with other
30 conventional anti-cancer drugs including radiation and novel methods of
delivery of angiostatin[®] to tumor cells (21). Mauceri et al (22) studied the
35 combined effect of radiation with angiostatin and showed that this
combination produced no increase in toxicity towards normal tissue. Both

in vitro and in vivo studies showed that these agents (radiation and
angiostatin[®]) in combination target the tumor vasculature. In an extension of
5 this study, Gorski et al (23) demonstrated that the efficacy of experimental
radiation therapy is potentiated by brief concomitant exposure of the tumor
vasculature to angiostatin[®].

10 Two novel methods of delivery of angiostatin and similar compounds to
the tumor cells that have been tried include:

15 (a) Nguyen et al (24) generated recombinant adeno-associated virus
(rAAV) vectors that carry genes encoding for angiostatin[®], endostatin[®],
20 and an antisense mRNA species against vascular endothelial growth
factor (VEGF). These rAAVs efficiently transduced three human tumor
cell lines that have been tested. Further, testing of the conditioned
25 media from cells transduced with this rAAV or with rAAV-expressing
endostatin[®] or angiostatin[®] inhibited effectively endothelial cell
30 proliferation in vitro. These results indicate that rAAVs can be used to
block angiogenesis and cancer growth.

35 (b) In a different approach, Chen et al (25) examined whether liposomes
complexed to plasmids encoding angiostatin[®] or endostatin[®] can inhibit

angiogenesis and growth of tumors. These studies revealed that plasmids expressing angiostatin[®] (PCI-angio) or endostatin[®] (PCI-endo) can effectively reduce angiogenesis and the size of the tumors implanted in the mammary fat pad of male mice to a significant degree. In addition, liposomes complexed to PCI-endo when given intravenously reduced tumor growth in nude mice by nearly 40% when compared to controls (25).

Summary of the Invention :

All the above factors and observations attest to the fact that malignant tumors are angiogenesis-dependent diseases. But, it should be mentioned here that tumor-associated angiogenesis is a complex, multi-step process which can be controlled by both positive and negative factors. It appears, as though, angiogenesis is necessary, but not sufficient, as the single event for tumor growth (26). But, it is evident from several experimental results that angiogenesis may be a common pathway for tumor growth and progression. Though several anti-angiogenic agents are being tried to arrest tumor growth, these are not without problems. Since the majority of these agents are proteins/peptides, their long-term use may lead to the development of antibodies which can neutralize their action. These anti-

angiogenic substances need to be given repeatedly and some of them are unstable and are difficult to produce in large amounts.

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In view of this, it is desirable and necessary to make efforts to stabilize and potentiate the actions of known anti-angiogenic molecules.

10

The present invention teaches the efficacious use of anti-angiogenic substances, which can inhibit endothelial cell proliferation and coupling them to cis-unsaturated fatty acids, which also have anti-angiogenic and cytotoxic actions on tumor cells, such that the actions of these substances are potentiated by each other. Further, as angiogenesis is involved in other disease processes such as inflammation, tumor metastasis, etc., it is envisaged that the conjugate(s) of anti-angiogenic substances and c-UFAs will be useful in these diseases also.

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In this context, it is important to note that the inventor has found that polyunsaturated fatty acids (PUFAs) such as gamma-linolenic acid (GLA), dihomogamma-linolenic acid (DGLA), arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can selectively kill the tumor cells ((27-32) and under specific conditions and in conjugation with salts such as lithium and a lymphographic agent these fatty acids can actually behave as anti-angiogenic substances, i.e. they block all the blood supply to the tumor

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and also prevent generation of new blood vessels. Using these fatty acids in this particular combination, the inventor has successfully treated human
5 hepatocellular carcinoma and giant cell tumor of bone with few or no side-effects.

10 Described hereinafter is a novel combination of a protein and a lipid and method(s) for its use. The protein referred to herein is a potent and specific
15 inhibitor of endothelial proliferation and angiogenesis. The lipid may be one or more of the polyunsaturated fatty acids: LA (linoleic acid), GLA, DGLA, AA, ALA (alpha-linolenic acid), EPA, DHA and cis-parinaric acid. In this
20 instance or method the polyunsaturated fatty acid need to be given only once or at the most twice within a period of 1 to 2 months. This invention teaches
25 that unlike angiostatin[®]/endostatin[®], these fatty acids are not only cytotoxic to the tumor cells but are also able to function as anti-angiogenic agents (33-
35). Further, polyunsaturated fatty acids when given in the formulated form,
30 are more potent than angiostatin/endostatin in their anti-angiogenic and anti-cancer actions.

35 The invention in one aspect teaches a method of interrupting blood supply to a tumor region causing necrosis or apoptosis. The invention also provides
40 a method of causing anti-angiogenic action in the tumor region with the

result that new blood vessels and collaterals are not formed to sustain the tumor. The present invention in another aspect tackles the issue of drug
5 delivery to the target tissue and provides the most efficacious method of administering an admixture of selected PUFAs with other elements such as
10 anti-angiogenic substances as will be described hereinafter.

The invention in yet another aspect teaches a method of interrupting
15 blood using a pre-determined admixture of at least a PUFA and an anti-angiogenic agent causing necrosis with very desirable results. Both the PUFAs and anti-angiogenic compounds being similar in function, the
20 invention also provides a method of causing anti-angiogenic action in the tumor region with the result that new blood vessels and collaterals are not
25 formed to sustain the tumor in the tumor region treated according to the invention. The present invention in another aspect tackles the issue of drug delivery to the target tissue and provides the most efficacious method of
30 administering an admixture of selected PUFAs along with an anti-angiogenic substance and other elements as will be described hereinafter.

35 Tumor cells are deficient in phospholipase A2, an enzyme necessary for the release of various PUFAs from the cell membrane lipids as a result of
40 which the production of anti-neoplastic PGs such as PGD2 are not

elaborated. In addition, tumor cells secrete an excess of PGE₂, an immunosuppressive and mutagenic substance. Further, tumor cells are deficient in PUFAs such as GLA, AA, EPA and DHA due to the low activity of delta -6-desaturase. As a result of these metabolic changes, tumor cells are able to effectively circumvent body's defense and survive. The present invention provides a method of causing necrosis of tumor cells despite their known survival pattern.

Anti-cancer actions of PUFAs:

Tumor cells are not only deficient in PUFAs but also have low rate(s) of lipid peroxidation, contain relatively large amounts of antioxidants such as vitamin E and superoxide dismutase (SOD). It is also believed that low rates of lipid peroxidation and consequent low amounts of lipid peroxides in the cells can contribute to an increase in the mitotic process which ultimately leads to an increase in cell proliferation. Thus, a deficiency of PUFAs, high amounts of antioxidants and the presence of low amounts of lipid peroxides in the tumor cells can contribute to the growth of tumor cells. This is supported by studies by the inventor wherein it was noted that PUFAs such as GLA, DGLA, AA, EPA and DHA can decrease tumor cell proliferation. In addition, it was also observed that when

appropriate amounts of GLA, DGLA, AA, EPA and DHA were administered to tumor cells and normal cells, obtained from American Type
5 Culture Collection, only tumor cells were killed without having any
significant action on the survival of normal cells in vitro. In mixed culture
experiments, in which both normal and tumor cells were grown together,
10 GLA showed more selective tumoricidal action compared to AA, EPA
and DHA though, these latter fatty acids were also effective to some
15 extent. This indicated that selective delivery of GLA, DGLA, AA, EPA
and DHA to tumor cells may offer a new therapeutic approach in the
treatment of cancer.

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These in vitro results are supported by in vivo studies performed in
animal tumor models. For example, it was noted that GLA, DGLA, AA,
25 EPA and DHA when used either in the form of pure fatty acid alone or in
the form of fatty acid rich oils could inhibit the growth of skin papilloma
30 in mice, formation and growth of hepatoma in rats and ascitic tumor cells
in the peritoneum of experimental animals. These results indicate that
these fatty acids can inhibit the growth of a variety of tumors even in vivo.
35 In further studies, it was noted that these fatty acids are able to enhance
free radical generation and the lipid peroxidation process selectively in the
40 tumor cells but not so much in the normal cells and thus, are able to

bring about their cancer killing action.

5 This ability of PUFAs to augment free radical generation and lipid
peroxidation in the tumor cells is analogous to the anti-tumor action of
lymphokines such as tumor necrosis factor (TNF) and interferon (IFN),
10 both alpha and gamma varieties. These lymphokines (also referred to as
cytokines) are capable of inducing the release of PUFAs from the cell
15 membrane lipid pool and enhance free radical generation in the cells.
Similarly several anti-cancer drugs such as, but not limited to,
doxorubicin and vincristine have the capacity to augment free radical
20 generation and promote lipid peroxidation. In addition, PUFAs and their
products can modulate immune response, augment a respiratory burst of
neutrophils and free radical generation by macrophages. This evidence is
25 further testified by the observation that the incidence of cancer in Eskimos
is low as influenced by their traditional diet, which is rich in EPA and
30 DHA. Inventor's studies have shown that PUFAs can be exploited as
possible anti-cancer agents either alone or in combination with
35 lymphokines and traditional anti-cancer drugs.

In a series of investigations by the inventor, it was also observed that the
40 cytotoxic action of anti-cancer drugs such as doxorubicin, vincristine and

cis-platinum can be augmented by various PUFAs such as GLA, DGLA, AA, EPA and DHA. In addition, these fatty acids could also enhance the cellular uptake of these anti-cancer drugs by the tumor cells and thus, are able to potentiate the anti-cancer actions of these drugs. In another similar experiment by the inventor, it was also observed that GLA, DGLA, AA, EPA and DHA were able to kill TNF resistant L-929 tumor cells in vitro. Further, these TNF-resistant tumor cells were rendered TNF sensitive by prior treatment of these L-929 cells by GLA, DGLA, AA, EPA and DHA. These results indicate that PUFAs can not only kill the tumor cells by themselves but are also capable of potentiating the cell killing effect of various anti-cancer drugs, lymphokines such as TNF and IFN and also render anti-cancer drug and TNF-resistant tumor cells sensitive to the cytotoxic action of various anti-cancer drugs and lymphokines.

In another set of experiments, it was also noted that vincristine resistant tumor cells, KB-^{ch-R} 8-5 (henceforth referred to as KB-8-5 cells) can be made sensitive to the cytotoxic action of vincristine by GLA, DGLA, AA, EPA and DHA. Further, when sub-optimal doses of vincristine and fatty acids were added together to these vincristine resistant cells produced optimal (i.e. significant) cell killing action. This shows that

vincristine and other anti-cancer compounds and PUFAs when added together to cancer cells, they potentiate the cytotoxic action of each other.

5 Fatty acid analysis of both vincristine sensitive (KB-3-1) and resistant (KB-8-5) cells revealed that the resistant cells have low amounts of GLA, AA, EPA and DHA compared to the vincristine sensitive tumor cells
10 indicating that a deficiency of these fatty acids may be responsible for their resistance to the cytotoxic actions of anti-cancer drugs. Since, both
15 vincristine sensitive and resistant tumor cells are easily (and to the same extent) killed by various PUFAs in vitro, this demonstrates that even
20 drug-resistant tumor cells can be killed by these fatty acids.

In yet another set of experiments, the inventor also noted that L-929
25 cells which are resistant to the cytotoxic action of tumor necrosis factor (referred to as TNF-resistant L-929 cells) can also be made sensitive to the cytotoxic action of TNF by pre-treating these cells with various
30 PUFAs. In other words, L-929 cells which are resistant to the cytotoxic action of TNF can be sensitized to the cytotoxic action of TNF by PUFAs. This again indicates that PUFAs can not only kill the tumor cells but can
35 also serve as sensitizing agents rendering various tumor cells responsive to the cytotoxic action of various anti-cancer drugs and lymphokines
40 (cytokines) such as tumor necrosis factor.

It is to be noted in this context that PUFAs can bind to albumin and other proteins and hence, if given intravenously may not be available to be taken up by the tumor cells and consequently may not be able to bring about their cell killing action on the tumor cells. In view of this, it is desirable that PUFAs including GLA should be delivered to the patients in such a manner that it is easily available to the tumor (tumor cells) and is delivered selectively to the tumor cells. It is highly desirable that PUFAs including GLA be given intra-tumorally as was experimentally done in the case of human gliomas, or, intra-arterially by selective intra-arterial infusion as was done experimentally in the case of hepatoma and giant cell tumor of the bone. But, it is also possible that in some cases of cancer such as Hodgkin's and non-Hodgkin's lymphoma wherein the tumor cells are extremely sensitive to the cytotoxic actions of PUFAs, even oral administration may be sufficient as was observed in certain patients. Since, PUFAs can potentiate the cell killing effect of anti-cancer drugs and lymphokines, it is desirable to administer a combination of PUFAs, anti-cancer drugs, lymphokines such as TNF and interferon or other anti-angiogenic agents or a combination thereof with or without a carrier agent such as an oily lymphographic agent as the situation indicates. Further

studies have also revealed that PUFAs such as GLA, DGLA and EPA can prevent or ameliorate the side effects of anti-cancer agents such as
5 gamma- radiation and cis-platinum to the bone marrow cells of mice.
Thus, it appears that when PUFAs and conventional anti-cancer
drugs/agents are given together they not only potentiate the cytotoxic
10 action of each on the tumor cells and thus, produce a synergistic
and/or additive action in their ability to eliminate the tumor cells but it
will also lead to elimination, reduction or amelioration of the side effects
15 of conventional anti-cancer agents. Since PUFAs are able to potentiate
the cytotoxic action(s) of conventional anti-cancer agents and Impho-
20 kines, it is also possible that this will lead to a significant reduction in the
doses of these latter agents without compromising the ultimate benefit
25 namely, elimination of tumor cells or the tumor.

Some of the phenomena which reduce the efficacy of the cytotoxic
30 action of PUFAs and conventional anti-cancer drugs/agents in vivo as
compared to in vitro results include the following:

- 35 a. PUFAs when administered orally or intravenously can bind to
albumin and other proteins in living beings and may not be available to

be taken up by the tumor cells. But this ability of PUFAs to bind to proteins is made use of in the present invention and is detailed below.

- 5 b. The cytotoxic action of PUFAs is produced by the augmentation of free radical generation and lipid peroxidation in only tumor cells (but not in normal cells). The intensity of the cytotoxic action is
- 10 disadvantageously reduced in actual clinical efforts because of inefficient transportation of the fatty acids to the target areas.
- 15 c. Continued blood supply to tissue with proliferative cell disorders is not conducive to bringing about a significant amount of necrosis
- 20 especially if the malignant cells multiply faster than they are being destroyed.
- 25 d. It was found from a study reported in a June, 1994 "Cancer letters" publication authored by N. Madhavi and U.N. Das that antioxidants
- 30 like vitamin E and the superoxide anion quencher, superoxide dismutase (SOD) could completely inhibit free radical generation and lipid peroxidation generated by PUFAs like GLA, EPA and
- 35 DHA. It appears that selective drug delivery to the target tissue will be conducive to the efficacy of the beneficial action of the PUFAs.
- 40 The present invention in one aspect resides in a method of inhibiting

blood supply to a tumor by using two types of substances: one a lipid
and the other a protein or a peptide both of which have very potent
5 anti-angiogenic action. In addition, the invention also comprises of the
steps of : locating an artery which carries major blood supply to the
tumor, said artery being one that is proximate to the tumor, and intra-
10 arterially injecting into the located artery a predetermined quantity of a
polyunsaturated fatty acid (PUFA) in the form of a solution of at least
one PUFA chosen from LA, GLA, DGLA, AA, ALA, EPA, DHA
15 and cis-parinaric acid in combination with a protein/peptide with
anti-angiogenic substance(s).

The invention in another aspect resides in a method for treating
tumors and for facilitating visualization of remission of the tumor
25 in response to treatment, comprising the steps of
(a) locating an artery which carries a major portion of blood supply to
30 the tumor and is adjacent to the tumor;
(b) obtaining an initial radiographic image of the tumor region;
(c) injecting into the artery a mixture of (i) an oily lymphographic
35 agent,
(ii) a lithium salt solution of at least one PUFA chosen from LA, GLA,
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DGLA, AA, ALA, EPA, DHA; and cis-parinaric acid

(iii) an anti-angiogenic protein/substance which is co-valently linked to
5 the fatty acid or form a mixture (fatty acid + anti-angiogenic
protein or peptide).

(d) obtaining second and subsequent radiographic images of the tumor
10 regions after predetermined lapses of time; and comparing the
initial radiographic images with the second and subsequent
15 radiographic images to assess the extent of remission of the tumor.

The invention in another aspect resides in a method of causing
20 necrosis in a cancerous tumor by inhibiting blood supply to the tumor,
and also by direct cytotoxicity to the tumor cells, comprising the steps
of :

25 (a) locating an artery proximate to the tumor which carries major blood
supply to the tumor;

30 (b) injecting into the located artery a mixture of (i) an anti-angiogenic
protein/peptide; (ii) a lithium salt solution of at least one
35 essential fatty acid chosen from LA, GLA, DGLA, AA, ALA, EPA,
DHA and cis-parinaric acid

(c) waiting for a predetermined time period and assessing a degree of necrosis in the tumor by examining by a radiographic study or by other means; and

(d) repeating step (b) if necessary to increase the necrosis.

In yet another aspect, the invention resides in a method of treating a glioma and visualizing remission of the glioma as it responds to treatment, comprising :

(a) obtaining an initial radiographic image of a region containing the glioma;

(b) injecting into the glioma region an admixture of (i) a sodium salt or any other suitable salt solution of at least one polyunsaturated fatty acid chosen from LA, GLA, DGLA, AA, ALA, EPA, DHA and cis-parinaric acid or a combination there of along with an anti-angiogenic protein/peptide;

(c) obtaining second and subsequent radiographic images of the glioma region after predetermined lapses of time; and comparing the initial radiographic pictures which shows the glioma , with second and

subsequent radiographic images of the glioma region to visualize

5 and assess the extent of remission of the glioma.

In yet another aspect, the invention resides in a method of treating
10 mammalian cell proliferative disorders using an emulsion of a lithium
salt of a PUFA or combinations of PUFAs and a predetermined anti-
15 angiogenic protein/peptide administered parenterally including a
subcutaneous route. Preferably, the intra-arterial administration of the
admixture containing PUFA(s) is done through a catheter. Also, the artery
20 carrying major blood supply to the tumor is to be understood herein as
synonymous to the artery which will supply the tumor feeding vessels.
Owing to a phenomenon which is consequent to inhibiting blood supply,
25 the present invention makes it not conducive to the formation of new
blood vessels i.e. angiogenesis. The anti-angiogenic protein in different
30 implementations of this invention may be endostatin[®] or angiostatin[®] or
any any other anti-angiogenic substance.

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Brief description of the illustrations

5 A more detailed understanding of the invention may be had from the following description of preferred embodiments, given by way of example, and to be understood in conjunction with the accompanying
10 illustrations/drawings wherein:

Figure 1 illustrates the structural metabolism of essential fatty acids.

15

Detailed description

20 Figure 1 shows a typical known metabolism pattern of essential fatty acids as known in prior art. Essential fatty acids are precursors of eicosanoids and are important structural components of cell membranes. They also provide the substrates for the generation of lipid peroxidation
25 products which have an inhibitory action on cell proliferation. Tumor cells are known to have low delta-6-desaturase activity, an enzyme necessary for the desaturation of dietary linoleic acid (LA, 18:2, n-6) and alpha-
30 linolenic acid (ALA, 18:3, n-3) to their respective products. In an earlier study, the inventor has shown that hepatocarcinogens, diethylnitrosamine (DEN) and 2-acetylaminofluorine (2-AAF), can suppress the activity of
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delta-6-desaturase and delta-5-desaturase resulting in low levels of
5 gamma-linolenic acid (GLA, 18:3, n-6) and arachidonic acid (AA, 20:4,
n-6) and eicosapentaenoic acid (EPA, 20:5, n-3) and docosahexaenoic
acid (DHA, 22:6, n-3) in the tumor cells. These results led the inventor and
10 others to study the effect of various fatty acids on the survival of tumor
cells in vitro. Addition of EFAs (LA and ALA) and other PUFAs such as
15 GLA, DGLA, AA, EPA, DHA and cis-parinaric acid to a variety of tumor
cells in vitro showed that only tumor cells are killed by these fatty acids
without harming the normal cells. This selective tumoricidal action of
20 fatty acids seems to be mediated by free radicals and lipid peroxides.
Similar to these fatty acids, radiation, some anti-cancer drugs and
25 cytokines (lymphokines) also seem to have the ability to generate free
radicals in tumor cells and thus, bring about their tumoricidal actions.

30 Since drug resistance is a major obstacle in the clinical treatment of
cancer and as PUFAs have selective tumoricidal action, the inventor
studied the effects of PUFAs on drug-resistant tumor cells and their
35 modulating influence on the actions of anti-cancer drugs.

In the above context, in addition to producing reversal of tumor cell drug resistance by the administration of polyunsaturated fatty acids, it is
5 seen from the invention that the manner of targeting the cancerous tissue is very critical to the efficacy and the speed with which necrosis can be brought about. More particularly, it is realized through this invention that
10 by delivering a chosen admixture of salts of predetermined polyunsaturated fatty acids and predetermined anti-angiogenic substance(s) to the
15 tumor site intra-arterially, intra-venously, subcutaneously, intra-peritoneally or by direct injection into the tumor bed, a very beneficial and hitherto unknown effect in terms of inhibiting blood supply to the tumor
20 site and inducing tumor cell lysis is achieved simultaneously.

25 In clinical studies conducted by the inventor with PUFAs, the inhibition of blood supply was pronounced enough to cause cutting off blood supply to the tumor site with very little time lag. In other instances, an
30 unmistakable strangling of blood supply to the tumor region was observed, but was relatively gradual.

35 One aspect of the invention consists in the preparation of a combination/composition of treatment of cancer in which one or more of LA, GLA,
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DGLA, AA, ALA, EPA, DHA and cis-parinaric acid are administered with conventional anti-cancer agents/drugs including anti-angiogenic protein/peptide with or without an oily lymphographic agent or any other suitable agent for the delivery of these compounds; optionally, radiation may be included. The PUFAs may be provided in a daily dose of 0.5 mg to 50 gm together with appropriate doses of conventional anti-cancer drugs such as vincristine, doxorubicin, L-asparaginase, cis-platinum, busulfan, etc., in a daily/weekly/monthly dose of 1 mg to 50 gm depending on the requirement and the stage of the disease and as may be determined from time to time with or without the addition of anti-angiogenic protein/peptide such as angiostatin[®]/endostatin[®] in a dose of 1 mg to 100 mg/kg of body weight per day. The word anti-angiogenic substance as used herein includes one or more of the following substances such as:

angiostatin[®], endostatin[®], platelet factor-4, TNP-470, thalidomide, interleukin-12, metalloprotease inhibitors (MMP), anti-adhesion molecules (in their desired dose). The combination of PUFAs, conventional anti-cancer drugs, anti-angiogenic substances and the

oily lymphographic agent may be administered by any one or different routes at the same time or at different times and intervals by selecting an appropriate route for each administration or in combination, eg. oral, parenteral including intra-arterial infusion, intravenous, subcutaneous, intra-peritoneal, topical, anal, vaginal routes as suppositories, or local injection directly into the tumor bed under the guidance of appropriate equipment such as but not limited to radiological guidance (X-rays), CT guidance or MRI guidance or by stereostaxic guidance. The daily dose(s) of these compounds may not exclude the administration of long acting preparations or depot preparation once or more times in a day, week, month or at some other appropriate time interval as determined from time to time depending on the necessity. The fatty acids (PUFAs) may be present in any physiologically acceptable form including but not limited to glycerides, esters, free acids, amides, phospholipids or salts. The conventional anti-cancer drugs may be administered by themselves or in conjugation with PUFAs (either alone or in combination such as GLA alone or GLA + AA, LA, DGLA, ALA, EPA or DHA). Similarly the anti-angiogenic substance(s) may be given by themselves or in

conjugation with PUFAs. For intra-arterial infusion or intravenous/
subcutaneous injection/infusion or administration of LA, GLA, DGLA,
5 AA, ALA, EPA, DHA and/or cis-parinaric acid these may be given by
themselves or in combination or dissolved or conjugated in/with anti-
10 angiogenic substances and in any other suitable solution that can be given
parenterally but not limited to them. All these PUFAs, conventional anti-
cancer drugs, anti-angiogenic substances and lymphographic agent may
15 each be given alone or in combination thereof or all together or separately
at the same time or at different time intervals on the same day/week
20 /month either by same route or different routes as the situation demands.

In order to observe or ascertain and record progress made in patients
25 after administration of admixture according to this invention, images of
the affected area eg., tumor region before and after treatment can be
obtained by various known modalities such as computerized axial
30 tomography (CT), magnetic resonance imaging (MRI), etc.

Examples:

1. Hard (wherein the PUFAs have been microencapsulated) or soft

gelatin capsules (wherein the fatty acids are present in an oily form)
made by accepted normal or forms or methods and are administered to
persons suffering from cancer in conjunction with conventional anti-
cancer drugs and/or anti-angiogenic substances in the doses as stated
supra.

2. Hard or soft gelatin capsules made by conventional methods, in which
the fatty acids, the anti-cancer drugs and anti-angiogenic substances
re incorporated together in the same capsule and are administered to
persons suffering from cancer.
3. As intra-tumoral preparation in appropriate doses (from 0.5 mg to 50
mg per day) of pure LA, GLA, DGLA, AA, ALA, EPA and DHA
either individually or in combination thereof especially with anti-
angiogenic substances for the treatment of human brain gliomas or
any other accessible tumor (eg. urinary bladder cancer, carcinoma
of the esophagus, carcinoma of the lung, breast cancer etc.) by any
route by using flexible fiber optic scopes such as bronchoscope,
urethroscope, hysteroscope, etc. In the case of tumors of the head
and neck the fatty acids are administered either by direct intra-

tumoral route or by selective catheterization of the tumor feeding vessel(s) either by femoral, brachial or carotid routes or by subcutaneous route or intravenous route. The PUFAs and anti-angiogenic substances can be given to these patients daily, weekly or monthly or as and when necessary depending on the requirement and response of the patient to the treatment.

4. Administered as selective intra-arterial infusion or injection into the tumor feeding vessel by femoral, brachial or carotid routes or any other suitable route or in a combination thereof the PUFAs either alone or in combination with anti-cancer drugs/anti-angiogenic substances with or without the oily lymphographic agent or any other suitable agent all in a mixture or in conjugated form(s) (like GLA + any conventional anti-cancer drug or drugs + anti-angiogenic substance, LA/GLA/DGLA/AA/ALA/EPA/DHA/cis-parinaric acid all individually or in combination thereof + conventional anti-cancer drug(s) + anti-angiogenic substance(s) + lymphographic agent., LA/GLA/DGLA/AA/ALA/EPA/DHA/cis-parinaric acid in combination with or conjugated to anti-angiogenic substance(s) or emulsified with or mixed with oily lymphographic agent.,

LA/GLA/DGLA/AA/ALA/EPA/DHA/cis-parinaric acid alone or
in combination thereof in oily lymphographic agent as a mixture
or emulsion or as a conjugate(s) and a variety of other combinations
thereof). This preparation may be administered daily, weekly or
monthly or at some other appropriate time interval.

5. Topical preparation of PUFAs either alone or in combination thereof
with conventional anti-cancer drugs or anti-angiogenic substance(s)
in a suitable delivery vehicle in which daily doses (ranging from 0.5 μ g
to 100 mg) are applied to primary skin cancers including Kaposi's
sarcoma locally and/or conventional anti-cancer drugs are given either
orally or parenterally.

By the different embodiments of the invention method described supra,
it becomes known that :

(i) when PUFAs or cis-EFAs (essential fatty acids described here
are also called as cis-fatty acids as by virtue of their structure are
referred to as cis-EFAs as they are in cis-configuration) are
administered to patients intra-arterially or even otherwise as a
combination with anti-angiogenic substance(s), there are less

chances of albumin and other proteins binding to the fatty acids.
Consequently, PUFAs thus administered using the invention are
5 better available to be taken up by the tumor cells.

(ii) Owing to the efficient transportation of PUFAs to the tumor site as
10 described hereinbefore, there is increased intensity of the cytotoxic
action of PUFAs and the administered anti-cancer agents (drugs or
15 anti-angiogenic substance(s) or a combination thereof). Thus, using
the invention, there is relatively better augmentation of free radical
generation and lipid peroxidation in the tumor cells, thereby
20 facilitating a greater degree of necrosis.

(iii) Inhibiting blood supply to the tumor region by the method of the
25 invention prevents cell proliferation in the tumor region, thus
enabling healthy tissue to grow back into place.

(iv) The inhibition otherwise caused by vitamin E and superoxide
30 dismutase to free radical generation and lipid peroxidation
35 produced by PUFAs, is reduced in the method of this invention because

of the manner of transportation of PUFAs to the tumor site in
combination with anti-angiogenic substance(s) intra-arterially
5 through a proximate artery or intravenously or subcutaneously.

It is also within the purview of this invention, as stated supra to admini-
10 ster an admixture of PUFAs, anti-cancer drugs, and selected anti-angiogenic
substance(s) at the same time, administering predetermined doses of PUFAs
orally. All such variations are envisaged to be within the ambit of this
15 invention.

20 **Application to mammals:** Even though the examples described supra relate
to humans, it is envisaged that the method of inhibiting blood supply and
using admixture of this invention including an anti-angiogenic substance are
25 equally applicable to other mammals.

30 Equivalents

While this invention has been particularly shown and described with
references to preferred embodiments thereof, it will be understood by those
35 skilled in the art that various changes in form and details may be made

therein without departing from the spirit and scope of the invention as
defined by the appended claims. For example, anti-angiogenic substances
5 referred to herein include not only Angiostatin[®] and Endostatin[®], platelet
factor-4, TNP-470, thalidomide, but other agents with anti-angiogenic
10 capabilities. Also sodium and potassium salts are considered equivalents of
each other. Imaging techniques referred to herein are intended to include
CAT, MRI, X-rays and other possible imaging methods. Those skilled in
15 the art will recognize or be able to ascertain using no more than routine
experimentation, many equivalents to the specific embodiments of the
20 invention described specifically herein. Such equivalents are intended to be
encompassed in the scope of the appended claims.

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Claims

- 5 1. A method of inhibiting blood supply to a tumor, comprising the steps
of:
- 10 (a) locating an artery which carries major blood supply to the
tumor, said artery being one that is proximate to the tumor ;
and
- 15 (a) intra-arterially injecting into the located artery a predetermined
quantity of a polyunsaturated fatty acid in the form of a solution
20 of at least one polyunsaturated fatty acid chosen from linoleic
acid, gamma-linolenic acid, dihomo-gamma-linolenic acid,
arachidonic acid, alpha-linolenic acid, eicosapentaenoic acid,
25 docosahexaenoic acid and cis-parinaric acid and one or more
anti-angiogenic substance(s).
- 30 2. A method as in claim 1 including the step of causing antiangiogenic
action, wherein polyunsaturated fatty acid is in the form of a lithium
35 salt solution and wherein said predetermined quantity of the fatty
acid is generally in a range of 0.5 mg to 50 gm.
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3. A method as in claim 1 wherein step (b) comprises intra-arterially
injecting a predetermined quantity of a polyunsaturated fatty acid in
the form of a derivative of a polyunsaturated fatty acid including at
least one predetermined anti-angiogenic substance to the extent of
1 to 1000 mg/kg/ body weight, said derivative of polyunsaturated
fatty acid being chosen from glycerides, esters, free acids, amides,
phospholipids and salts.
4. A method as in claim 1 wherein the polyunsaturated fatty acid is in
the form of a lithium salt solution of gamma-linolenic acid and
eicosapentaenoic acid/docosahexaenoic acid, including a predeter-
mined quantity of anti-angiogenic substance chosen from
Angiostatin[®], Endostatin[®], platelet factor-4, TNP-470, thalidomide,
interleukin-12, and metalloprotease inhibitors, and a predetermined
anti-cancer drug.
5. A method of treating a tumor and facilitating visualization of
remission of the tumor responsive to treatment, comprising
- (a) locating an artery which carries a major portion of blood supply to

said tumor and is adjacent to the tumor;

(b) obtaining an initial radiographic image of the tumor region;

(c) injecting into the located artery a mixture of at least

(i) an oily lymphographic agent as a carrier containing one or more
of anti-angiogenic substance(s)

(ii) a lithium salt solution of at least one polyunsaturated fatty acid
chosen from linoleic acid, gamma-linolenic acid, dihomo-gamma-
linolenic acid, arachidonic acid, alpha-linolenic acid,
eicosapentaenoic acid, docosahexaenoic acid and cis-parinaric
acid

(d) obtaining second and subsequent radiographic images of the tumor
region after predetermined lapses of time; and

(e) comparing the initial radiographic image with the second and
subsequent images to assess an extent of remission of the tumor.

6. A method as in claim 5 wherein step (c) comprises intra-arterially
injecting a mixture containing an anti-angiogenic substance chosen

from Angiostatin[®], Endostatin[®], platelet factor-4, TNP-470,
5 thalidomide, interleukin-12 causing anti-angiogenic action by
inhibiting the blood supply to the tumor, wherein further the oily
lymphographic agent acts as a carrier for anti-angiogenic sub-
10 stance(s), and also for the lithium salt solution of predetermined
quantities of gamma-linolenic acid, eicosapentaenoic acid and/or
15 docosahexaenoic acid.

7. A method of treating a glioma and visualizing remission of the
20 glioma as it responds to treatment, comprising:

(a) obtaining an initial radiographic image of a region containing the
25 glioma;

(b) injecting into the glioma region an anti-angiogenic substance and a
30 sodium salt of at least one polyunsaturated fatty acid chosen from
linoleic acid, gamma-linolenic acid, dihomo-gamma-linolenic acid,
arachidonic acid, alpha-linolenic acid, eicosapentaenoic acid,
35 docosahexaenoic acid and cis-parinaric acid which are (is) co-valently
linked to said anti-angiogenic substance.

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(c) obtaining second and subsequent radiographic images which show the glioma region after predetermined lapses of time; and

5 (d) comparing the initial radiographic image which shows the glioma, with second and subsequent radiographic images of the glioma region to visualize and assess an extent of remission of the glioma.

10 8. A method as in claim 7 wherein the sodium salt solution comprises a solution of predetermined quantities of sodium salt of at least one polyunsaturated fatty acid and one or more of anti-angiogenic substance(s) chosen from Angiostatin[®], Endostatin[®], platelet factor-4, TNP-470, thalidomide, and interleukin-12.

25 9. A method of treating a cancerous tumor, comprising

(a) using an oily lymphographic agent as a carrier for

30 (i) at least one polyunsaturated fatty acid chosen from a lithium salt of at least one of linoleic acid, gamma-linolenic acid, dihomo-gamma-linolenic acid, arachidonic acid, alpha-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid and cis-parinaric acid

(ii) one predetermined anti-cancer drug, and anti-angiogenic
substance(s) which are mixed with polyunsaturated fatty acids
or co-valently linked to fatty acids

(b) administering a predetermined quantity of selected fatty acids and
predetermined anti-angiogenic substance in the oily
lymphographic agent as a carrier.

10. A method of treating a mammalian cell proliferative disorder including
cancer with a polyunsaturated fatty acid such as gamma-linolenic acid,
arachidonic acid, eicosapentaenoic acid or docosahexaenoic acid or
cis-parinaric acid or combinations thereof, comprising the steps of :
preparing the polyunsaturated fatty acid in the form of a lithium salt
in an emulsion with an oily lymphographic agent including covalent
conjugation with a pharmaceutical agent chosen from vincristine,
adriamycin, doxorubicin, cyclophosphamide, cis-platinum, L-
asparaginase, procarbazine, camptothecin, taxol or busulfan; and
at least one anti-angiogenic substance and administering said emulsion
parenterally.

11. The method of claim 10 wherein the anti-angiogenic substance in the emulsion includes at least one biological compound chosen from Angiostatin[®] and Endostatin[®] which is naturally occurring in the body and which has an anti-cancer action, said method including the step of using said biological compound as an anti-angiogenic substance.
12. The method of claim 10 wherein the oily lymphographic agent is used as a carrier.
13. The method of claim 10 wherein the disorder is any type of cancer including but not limited to hepatoma, bronchogenic cancer of the lung, colon cancer, breast cancer, ovarian cancer, cancer of the kidney such as hypernephroma, skin cancer such as melanoma, Kaposi's sarcoma, cancer of the esophagus, cancer of the stomach, leukemias of all types or lymphomas of all types and wherein the emulsion includes a predetermined quantity of an anti-angiogenic substance, chosen from Angiostatin[®], Endostatin[®], platelet factor-4, TNP-470, thalidomide, interleukin-12, and metalloproteinase inhibitors (MMP).

invention include Angiostatin[®], Endostatin[®], platelet factor-4, TNP-470,
5 thalidomide, interleukin-12 and metalloproteinase inhibitors (MMP). A
preferred method of administration of the mixture to treat a tumor is intra-
10 arterial administration into an artery which provides the main blood supply
for the tumor.

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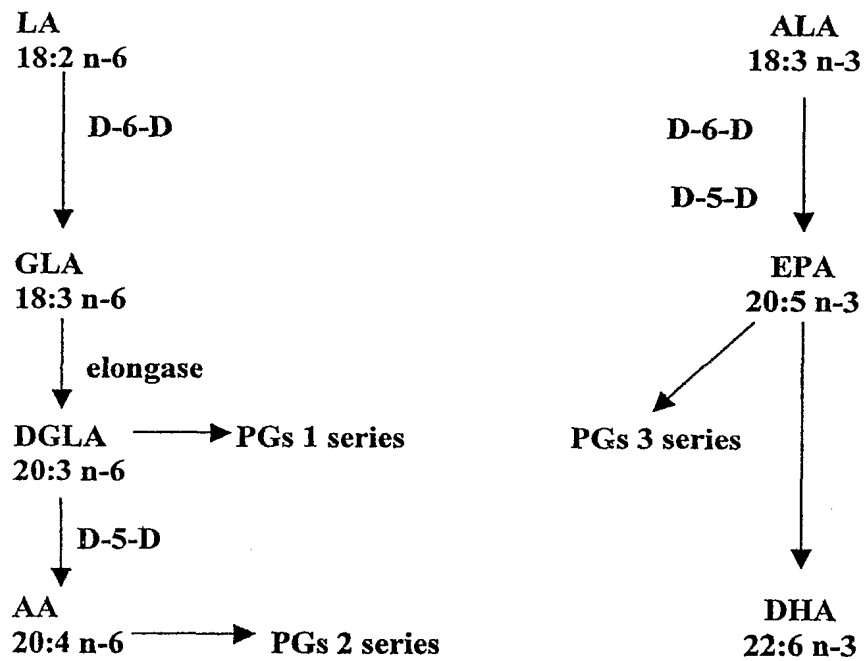


FIGURE 1 SHOWING THE METABOLISM OF ESSENTIAL FATTY ACIDS

PRIOR ART

FIGURE 1

INTERNATIONAL SEARCH REPORT

Internat'l Application No

PCT/US 00/01037

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 A61K31/20 A61K45/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EPO-Internal, BIOSIS, EMBASE, MEDLINE, CANCERLIT, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YAMAMOTO, DAIGO ET AL: "Synergistic action of apoptosis induced by eicosapentaenoic acid and TNP - 470 on human breast cancer cells" BREAST CANCER RES. TREAT. (1999), 55(2), 149-160, XP000957580 abstract	1
Y	WO 98 09621 A (SCOTIA HOLDINGS PLC ;SCOTT CATHERINE ANN (GB); HORROBIN DAVID F (G) 12 March 1998 (1998-03-12) page 5, paragraph 3 -page 6, paragraph 1; claims -/--	2,4-10

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

30 October 2000

Date of mailing of the international search report

08/11/2000

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Leherte, C

INTERNATIONAL SEARCH REPORT

Intern: I Application No

PCT/US 00/01037

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 795 909 A (SHASHOUA VICTOR E ET AL) 18 August 1998 (1998-08-18)	1,3
Y	column 19, line 62 - line 64 column 31, line 46 column 33, line 23 column 40, line 61 column 44, line 13 column 45, line 57 column 47, line 41 - line 51 -----	2,4-10

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-13

Present claims 1-13 relate to compositions defined (inter alia) by reference to the following parameters namely "polyunsaturated fatty acid", "anti-angiogenic agent", "metalloprotease inhibitor", "anti-cancer drug" or "lymphographic agent".

The use of these parameters in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is not fully possible to compare the parameters the applicant has chosen to employ with what is set out in the prior art. The lack of clarity is such as to render a meaningful complete search impossible. Consequently, the search has been restricted to those parts relating to the compounds specially mentioned in claim 7, with due regard to the general idea underlying the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern: I Application No

PCT/US 00/01037

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9809621 A	12-03-1998	AU 4124997 A BR 9711671 A CN 1235542 A EP 0956012 A ZA 9707902 A	26-03-1998 18-01-2000 17-11-1999 17-11-1999 11-08-1998
US 5795909 A	18-08-1998	AU 3142597 A EP 0909183 A WO 9744063 A	09-12-1997 21-04-1999 27-11-1997